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Summer seasonal prevalence of *Culicoides* species from pre-alpine areas in Switzerland

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Abstract. Biting midges (Diptera: Ceratopogonidae) are arthropods of veterinary importance since they can transmit pathogens and cause severe allergic dermatitis in horses. Very little is known about the species at higher altitudes and their seasonal dynamics. In this work, adult *Culicoides* were collected with Onderstepoort UV-light suction traps (OVI) from June to September 2016 at two areas situated at around 1600 m asl (pre-alpine area I, 2 farms) and 2030 m asl (pre-alpine area II, 1 farm) in the Canton of Grisons (south-east Switzerland). Overall, 17 049 *Culicoides* were collected, including 871 parous females. A total of 50 individuals/trap/night (n = 1050) were identified to species (17 species) by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) or by polymerase chain reaction (PCR) and sequencing. The remaining 15 128 *Culicoides* were classified to species groups' level. *Culicoides obsoletus* (Meigen, 1818), a multivoltine species, was mainly present at 1600 m asl, whereas at high altitudes (2030 m asl), *C. grisescens* Edwards, 1939 I&II were the most abundant species. In particular, *C. grisescens II*, which seems to be univoltine, occurred later in the season but significantly increasing over time. Species diversity was higher at pre-alpine I area (n = 16 species) compared to pre-alpine II (n = 10 species).

Key words. Animal health, biting midges, cattle, high altitudes, species, vectors.

Introduction

Culicoides biting midges (Diptera: Ceratopogonidae) are among the world's smallest haematophagous flies reaching up to 3 mm in size. Currently, there are more than 1400 identified species (Borkent & Wirth, 1997), and their presence has been recorded worldwide with the exception of Antarctica and New Zealand, ranging from the tropics to the tundra and from sea level to 4000 m above sea level (m asl) (Mellor *et al.*, 2000). These arthropods are important vectors of pathogens affecting both animal and human health (Carpenter *et al.*, 2013), including viruses (Mellor *et al.*, 2000), protozoa (Bukauskaite *et al.*, 2015), nematodes and haemosporidians (Linley *et al.*, 1983; Martinez-de la Puente *et al.*, 2011). In addition, they cause severe allergic dermatitis mainly in horses (Braverman *et al.*, 1983).

The arboviruses of current interest in Europe, which have been confirmed to be transmitted by *Culicoides*, are bluetongue virus (BTV) (Du Toit, 1944) and Schmallenberg virus (SBV) (De Regge *et al.*, 2012). Biting midges are also the biological vectors of African horse sickness virus (AHSV) and epizootic haemorrhagic disease virus (EHDV) which are considered a risk for Europe (Zientara *et al.*, 2015; Robin *et al.*, 2016). The *Culicoides* species vectors or potential vectors of BTV, specifically in central and northern Europe, are *C. obsoletus* and *C. scoticus* Downes and Kettle, 1952 from the Obsoletus complex, *C. dewulfi* Goetghebuer, 1936, *C. chiopterus* (Meigen, 1830) from the Obsoletus group, *C. pulicaris* (Linnaeus, 1758) and *C. punctatus* (Meigen, 1804) (De Liberato *et al.*, 2005; Saegerman *et al.*, 2008) from the Pulicaris group, while the putative SBV vectors are species within the Obsoletus complex (*C. obsoletus* and *C. scoticus*), *C. dewulfi* and

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C. chiopterus species (De Regge *et al.*, 2012). While female specimens from the Obsoletus complex are not morphologically distinguishable, the Pulicaris and Obsoletus groups exhibit a notable degree of morphological differentiation (Harrup *et al.*, 2015).

Studies from Switzerland have revealed that species of the Obsoletus group (Kaufmann *et al.*, 2012b) are predominant (>95% of collected *Culicoides*) up to altitudes of around 1100 m asl (Kaufmann *et al.*, 2009), whereas at higher altitudes, species of the Pulicaris group (*C. deltus* Edwards, 1939, *C. grisescens*, *C. impunctatus* Goetghebuer, 1920, *C. lupicaris* Downes and Kettle, 1952, *C. newsteadi* Austen, 1921, *C. pulicaris*, *C. punctatus*) (Kaufmann *et al.*, 2012b) are more abundant, accounting for 50% and 80% of the biting midges populations at 1300 and 2000 m asl, respectively (Kaufmann *et al.*, 2009, 2012a).

Interestingly, species determination by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) of 100 randomly selected individual *Culicoides* specimens, collected over one night in late summer (period of highest abundance and highest probability for pathogen transmission) revealed that the Pulicaris group species *C. grisescens*, which contains two cryptic (morphologically indistinguishable) species (Wenk *et al.*, 2012; Kaufmann *et al.*, 2012b), dominates the midge fauna at higher altitudes in Switzerland (Kaufmann *et al.*, 2012a). Another five *Culicoides* species were occasionally identified in samples from locations >1500 m asl in that study. These included the Obsoletus complex species and single specimens of *C. fascipennis* (Staeger, 1839), *C. pallidicornis* Kieffer, 1919 and *C. reconditus* Campbell and Pelham-Clinton, 1960, that do not belong to either Obsoletus or Pulicaris group, but described as other *Culicoides* species. *Culicoides grisescens* was sporadically found at lower altitudes in Switzerland (Kaufmann *et al.*, 2012b) and to some extent in northern European countries (Denmark, The Netherlands, Sweden, e.g. representing 3% in the Swedish study) (Ander *et al.*, 2012; Meiswinkel *et al.*, 2014).

Epidemiological data from Switzerland have shown that BTV, whose transmission was eliminated at an early stage by vaccination campaigns (Willgert *et al.*, 2011), was not circulating among livestock at higher altitudes. However, investigations of free-ranging ruminants revealed a PCR-positive chamois originating from the Engadin valley, at 2000 m asl (Casaubon *et al.*, 2013). Furthermore, the highest altitude where a proven transmission of SBV has occurred in Switzerland is Lenzerheide (canton Grisons) at around 1500 m asl (Dr C. Nathues, Federal Food Safety and Veterinary Office, Bern, Switzerland, personal communication). Thus, transmission of *Culicoides*-borne viruses at higher altitudes appear possible, but is not clear which *Culicoides* species are involved nor whether such transmission could regularly occur under average summer conditions or only exceptionally under warm conditions.

Biting midges in Switzerland have been studied in the last years (Casati *et al.*, 2009; Kaufmann *et al.*, 2009) but there is a lack of information regarding their biology and ecology, particularly at higher altitudes (Kaufmann *et al.*, 2012a) this also in relation to vector season at these altitudes. According to The European Commission, the start of the vector season is termed as the week during which the number of parous females caught exceed a threshold (five for *C. obsoletus* and one for *C. imicola* Kieffer, 1913) (Commission, 2007).

The aim of our work was to determine prevalence of *Culicoides* species during the summer season at two pre-alpine areas. Moreover, the presence and abundance of *Culicoides* species which are known for their role in pathogens transmission are here presented.

Materials and methods

Study sites

Culicoides specimens were caught in 2016 at three farms (Table 1) situated in the pre-alpine region, Canton of Grisons (south-east Switzerland). Two farms were located at altitudes of 1575 and 1650 m asl (Davos Wolfgang and Davos Clavadel, approx. 7 km as the crow flies), further called pre-alpine I area, and one farm (Davos Clavadeler Alp) at an altitude of 2030 m asl, further called pre-alpine II area. All farms were located near a forest and the one in Davos Wolfgang was also in close vicinity with a lake. Piles of open-air animal manure were present at each farm. More than one category of animal was present at each farm (Table 1) except for the farm from Davos Clavadel where only cattle were present although by the end of June 2016, these were moved higher up into the mountains (Davos Clavadeler Alp). This is part of the transhumance, moving livestock from one grazing ground to another in a seasonal cycle, often practiced in the Swiss mountains.

Climatic data

We used the data provided by the Federal Office of Meteorology and Climatology (Meteoswiss) through IDAWEB personal registration. Climatic data were recorded from the closest meteorological stations to our pre-alpine studied areas: Davos Stilli (1560 m asl) 46° 48′ 45″N, 09° 50′ 50″E (pre-alpine I) and Davos Parsenn (2290 m asl) 46° 51′ 06″N, 09° 48′ 17″E (pre-alpine II).

Trapping methods and Culicoides collection

Adult *Culicoides* were trapped using Onderstepoort UV-light suction traps (OVI) (Venter *et al.*, 1996). Traps were run on 220 V, and biting midges were collected alive in cubic netted cages situated below the suction fan (17.5 × 17.5 × 17.5 cm; BugDorm-42222F; MegaView Science Co., Ltd., Taichung, Taiwan). A wet paper was added to the bugdorm to avoid insect desiccation.

Traps were activated from 3rd of June to 3rd of October at each farm at irregular intervals when suitable conditions occurred, e.g. no storms, no heavy wind speed or night temperatures ≥8 °C (total 12 collections), and operated from approximately 1–2 h before sunset to 1–2 h after sunrise of the following day. Bugdorms were transported to the laboratory where *Culicoides* were immediately removed using a mouth aspirator and stored in 50 mL 70% ethanol at 4 °C until further identification.

Table 1. Areas and features of the *Culicoides* trapping locations.

Area	Farm (altitude, m asl)	Location, GPS coordinates of trap	Domestic animals present
Pre-alpine I	Davos Wolfgang (1575)	Davos Wolfgang 46°49′26.30″N 9°51′34.90″E	Sheep (≤30), pigs (≤5), rabbits (≤4), and chickens (≤10)
	Davos Clavadel (1650)	Davos Clavadel 46°46′07.14″N 9°48′44.02″E	Cattle (≤10)
Pre-alpine II	Davos Clavadel (2030)	Davos Clavadeler Alp 46°46′27.40″N 9°49′28.65″E	Cattle (≤30) and pigs (≤5)

Table 2. Number* of *Culicoides* and parous *Culicoides* collected with Onderstepoort UV-light suction traps in seven collection nights (between 30th June and 14th September 2016) in two Swiss pre-alpine areas.

Area	Trap location	No. of collected *Culicoides**/ median (range)	No. of parous females / median (range)
Pre-alpine I (around 1600 m asl)	Davos Wolfgang farm	5828/93.5 (0–3758)	322/7 (0–144)
	Davos Clavadel farm	1614/35 (0–640)	60/1 (0–34)
Pre-alpine II (2030 m asl)	Davos Clavadeler Alp farm	8736/64 (0–5594)	489/0 (0–360)
Total Pre-alpine I&II		16 178	871

*Including also the 1050 individuals used in the species identification study.
Median and range are calculated per trap/night.

Culicoides identification

First, the parous (pigmented) *Culicoides* were counted and identified under a stereo-microscope (Dyce, 1969) and species sorted as Obsoletus group, Pulicaris group and other *Culicoides* species for the specimens that do not belong to the two groups based on wing pattern and morphological features using the key described (Mathieu *et al.*, 2012). From the rest of the catches, due to the limited budget of our study only subsamples were tested for species identification. We randomly picked 50 *Culicoides* from each night collection/farm/area and determined the species mainly by MALDI-TOF MS as described (Kaufmann *et al.*, 2012a). Samples with unclear results were also analysed by PCR/sequencing (Wenk *et al.*, 2012).

The remaining *Culicoides* were further counted and divided as: Obsoletus group (including *C. chiopterus*, *C. obsoletus*, *C. scoticus* species), Pulicaris group (including *C. deltus*, *C. grisescens* I&II (Wenk *et al.*, 2012), *C. lupicaris*, *C. pulicaris*, *C. punctatus*), and other *Culicoides* species (including *C. brunnicans* Edwards, 1939, *C. comosioculatus* Tokunaga, 1956, *C. fascipennis*, *C. furcillatus* Callot, Kremer and Paradis, 1962, *C. jurensis* Callot, Kremer and Deduit, 1962, *C. pallidicornis*, *C. segnis* Campbell and Pelham-Clinton, 1960, *Culicoides* sp. (Wenk *et al.*, 2012; Kaufmann *et al.*, 2012b). For catches of more than 500 individuals, a modified version of the sub-sampling procedure described by Van Ark & Meiswinkel (1992) was used: the *Culicoides* sample in 70% ethanol was poured into a Petri dish, 1 mL/catch was analysed and the total number of insects extrapolated. Only female *Culicoides* were considered and counted for the study because males do not play a role as vectors or biting nuisance as they do not blood feed. Furthermore, they only constitute a small proportion of adults caught in the light traps (Purse *et al.*, 2012).

Statistical analyses

A logistic regression analyses was applied to assess whether the proportions of each *Culicoides* species collected varied according to area and time period during the 2016 summer season. Using logistic regression (i.e. the proportion of each species found at each area on each date) we transformed dates into time (in days) from the first observation (30th June 2016, the first date with collection of *Culicoides*). Time then becomes a continuous variable. All analyses take into account the total number of *Culicoides* examined. The analyses were performed using R (https://www.r-project.org/).

Results

Culicoides *collection*

Overall, a total of 17 049 *Culicoides* were collected at the three farms in the pre-alpine areas I&II across 7 of the 12 trapping nights (Table 2). Out of these, 871 (5% of total midges) were parous (Table 2, Fig. 1A,B), 1050 were analysed to species level (Table 3), whereas the remaining 15 128 were identified to group level. No *Culicoides* were collected in five nights for both trapping areas due to heavy snow or low temperatures. In those specific nights, the lowest temperatures were recorded in both areas (0.3–6.9 °C at pre-alpine I, and −2.9–2.9 °C at pre-alpine II see Table S1).

Climatic data

The mean daily temperature recorded during collection nights at the pre-alpine I area ranged between 3.7 and 15.9 °C, whereas

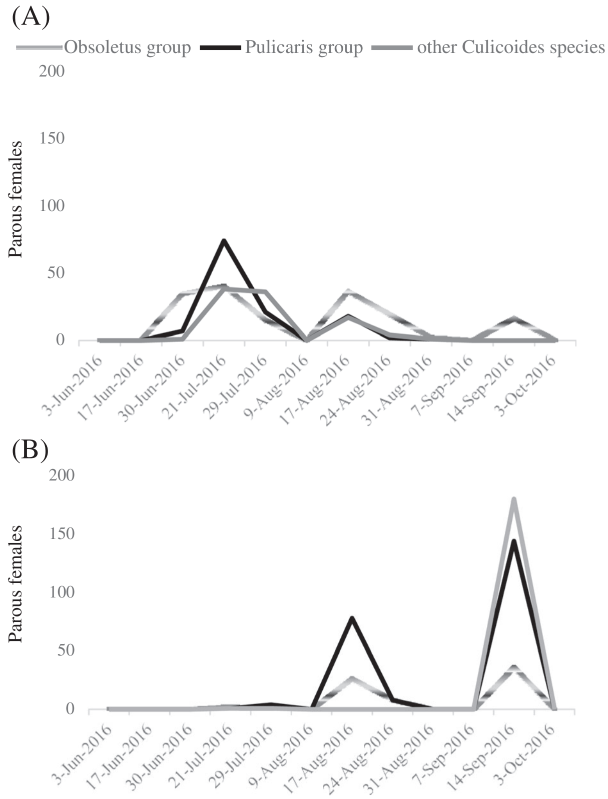

Fig. 1. Parous *Culicoides* (sorted to species group level) collected in June–September 2016 in (A) pre-alpine I (around 1600 m asl; two farms with one trap each) and (B) pre-alpine II (2030 m asl; one farm) areas.

the mean daily precipitation ranged between 0.6 and 20.7 mm (Fig. 2A). The daily mean wind speed fluctuated among 1 and 2.6 m/s. In the pre-alpine II area, the daily mean temperature registered was between −1.2 and 15.6 °C, daily mean precipitation between 0.3 and 39.8 and wind speed 1.1–2.1 m/s (Fig. 2B). The highest daily mean temperature during our collection nights was recorded on the 21st of July at the pre-alpine I area (16.1 °C) and 24th of August at the pre-alpine II area (15.6 °C). The lowest daily mean temperature was observed at our last collection day in both areas, on the 3rd of October (3.7 °C at pre-alpine I and −1.2 °C at pre-alpine II areas, respectively). Three peaks of precipitation were recorded in the pre-alpine I (3rd June, 21st July and 9th August) and four in the pre-alpine II area (17th of June, 21st of July, 17th and 31st of August).

However, there were six collection dates with no precipitation registered at the pre-alpine I area (including the last five collection days in a row) and 3 days in the pre-alpine II area.

The maximum mean wind speed registered during the study was higher in the pre-alpine I area (2.6 m/s on the 29th of July) compared with the pre-alpine II area (2.1 m/s on the 14th of September). The climate data registered (daily mean of: T °C, wind speed (m/s) and precipitations (mm)) during *Culicoides* collection are expressed in Fig. 2A,B. The maximum and minimum temperatures recorded during collection nights are provided in Table S1.

Parous *Culicoides*

Among the 871 parous *Culicoides* collected during the 2016 summer season, the majority were found in the pre-alpine II area (n = 489) (Fig. 1B) with the first individuals (n = 4) collected by end of July. The presence of more than five parous midges in this area was recorded by mid-August (n = 104) mainly with those from the Pulicaris group (n = 78) (Fig. 1B) followed by a second peak in mid-September (n = 360), where other *Culicoides* species were slightly more abundant (n = 180).

At the pre-alpine I area (Fig. 1A), the first parous midges were observed end of June (n = 42) with the Obsoletus group species being predominant (n = 34) (Fig. 1A). Parous *Culicoides* in this area were collected until mid-September, with 16 specimens from the Obsoletus group. Three peaks were registered during the summer season with the highest one observed by the end of July.

Summer seasonal prevalence of Culicoides species

Out of the 1050 biting midges that we selected for species identification, 1014 (673 from the pre-alpine I and 341 from the pre-alpine II area) were successfully identified down to species level by MALDI-TOF MS or PCR/sequencing (Table 3). The remaining 36 specimens for which it was not possible to identify the species since their mass profiles (MALDI-TOF MS identification) and sequences are not included in the respective databases, we here referred to as unknown species. Thus, 17 *Culicoides* species, including the two cryptic species of *C. grisescens*, were identified (Table 3).

In the pre-alpine I farms, the overall predominant species were *C. obsoletus* (30%) and *C. pulicaris* (20%) while in the pre-alpine II farm the main catches were *C. grisescens* II (41%) and *C. pulicaris* (30%) (Table 3).

Culicoides obsoletus was present throughout the summer until mid-September at the pre-alpine I area (≤1650 m asl), whereas at the pre-alpine II area (2030 m asl), it appeared only in one collection (n = 2) at the beginning of the season (late June) (Table 3). *Culicoides scoticus*, belonging to the Obsoletus complex was found at the pre-alpine I area only, without any tendency over the whole studied period.

The proportion of *C. pulicaris*, present at both areas (pre-alpine I&II) decreased over the season (z value = −5.6 and $P < 0.001$), and there was no effect of area. *Culicoides grisescens* I&II were both more frequent at the higher altitude area ($P < 0.001$). Moreover, *C. grisescens* I, which was overall less abundant than the other cryptic species *C. grisescens* II, was collected over the whole summer season from June to September. *Culicoides grisescens* II appeared only 1 month later but increased over the time (positive parameter, highly significant, z value = 10.2 and $P < 0.001$) (Table 3).

Culicoides deltus, was sampled at both altitudes in similar abundances, with no significant statistic variation according to the season or the area sampled. For *C. punctatus*, there was a decrease in the proportion over the summer (z value = −2.4 and $P = 0.01$), whereas *C. pallidicornis* was found in greater proportion at pre-alpine I area (z value = −2.2 and $P = 0.01$), and there was no effect of time of season.

Table 3. Mondrian matrix of the *Culicoides* collected at the pre-alpine I (around 1600 m asl; two sites with one trap each) and pre-alpine II (2030 m asl; one site) areas during the summer season 2016.

(a) Trapping date Species/area	03.06. 2016 I	17.06. 2016 I	30.06. 2016 I	21.07. 2016 I	29.07. 2016 I	09.08. 2016 I	17.08. 2016 I	24.08. 2016 I	31.08. 2016 I	07.09. 2016 I	14.09. 2016 I	03.10. 2016 I	Total n (%)
Culicoides obsoletus			45	14	32		30	25	21		45		212 (30)
C. grisescens II					6		3	11	22		35		77 (11)
C. pulicaris			29	43	24		27		15		3		141 (20)
C. deltus			1	9	19		5	17	16				67 (10)
C. scoticus			17	13	2		17	9	16		2		76 (11)
C. grisescens I			3	2	1		8	20	1		2		37 (5)
Unknown spp.*				7			2	10			8		27 (4)
C. pallidicornis				3	8		6	3	2		2		24 (3)
C. lupicaris					2		2		2		3		9 (1)
C. fascipennis			2	3	3				1				9 (1)
C. punctatus			3	3				1	1				8 (0.1)
Culicoides sp.†								4					4 (0.5)
C. comosioculatus				1	2				1				4 (0.5)
C. jurensis									2				2 (0.2)
C. chiopterus				2									2 (0.2)
C. brunnicans													0 (0)
C. furcillatus					1								1 (0.1)
C. segnis													0 (0)
Culicoides tested	0	0	100	100	100	0	100	100	100	0	100	0	700

(b) Trapping date Species/area	03.06. 2016 II	17.06. 2016 II	30.06. 2016 II	21.07. 2016 II	29.07. 2016 II	09.08. 2016 II	17.08. 2016 II	24.08. 2016 II	31.08. 2016 II	07.09. 2016 II	14.09. 2016 II	03.10. 2016 II	Total n (%)
Culicoides obsoletus			2										2 (0.5)
C. grisescens II					4		26	27	48		40		145 (41)
C. pulicaris			35	15	32		8	13			2		105 (30)
C. deltus			7	14	5		6	4	1		2		39 (11)
C. scoticus													0 (0)
C. grisescens I			5	8	9		9	4	1		6		42 (12)
Unknown spp.*				6			1	2					9 (3)
C. pallidicornis				3									3 (0.8)
C. lupicaris													0 (0)
C. fascipennis													0 (0)
C. punctatus													0 (0)
Culicoides sp.†				3									3 (0.8)
C. comosioculatus													0 (0)
C. jurensis													0 (0)
C. chiopterus													0 (0)
C. brunnicans				1									1 (0.2)
C. furcillatus													0 (0)
C. segnis				1									1 (0.2)
Culicoides tested	0	0	50	50	50	0	50	50	50	0	50	0	350

*No corresponding profile or sequence in mass spectrometry database or GenBank.
†Novel *Culicoides* species identified by PCR/sequencing by Wenk *et al.*, 2012 (GenBank accession nr. HQ824436.1).
Fifty randomly chosen *Culicoides* individuals were identified to species level by MALDI-TOF MS or PCR/sequencing per night/trap farm: Table (a) pre-alpine I, 2 farms; Table (b) pre-alpine II, 1 farm. *Colour code*: white = 0 *Culicoides*, dark white = 1–9, light grey = 10–19, grey = 20–29, dark grey = 30–39, black = 40–50.

For the rest of the collected *Culicoides* species (*C. brunnicans*, *C. comosioculatus*, *C. chiopterus*, *C. fascipennis*, *C. furcillatus*, *C. jurensis*, *C. lupicaris*, *Culicoides* sp., and *C. segnis*), there was no significant statistic variation according to the summer season or the area sampled (Table 3).

Culicoides *groups*

Overall, out of the 15 128 specimens collected at the pre-alpine I&II areas, respectively, 3716 (25%) and 4090 (27%) belonged to the Pulicaris group, 1710 (11% and 1356 (9%) to the Obsoletus group and 1316 (9%) and 2940 (19%) to the other *Culicoides* species.

The first *Culicoides* at the pre-alpine I area were collected by end of June (n = 953), and the peak activity was rapidly reached in mid-July, with species of the Pulicaris group being predominant (n = 2407). Thereafter, the activity decreased until mid-August, interrupted by a period of no activity (Fig. 2A).

The number of midges collected at the two farms differed considerably, both with regard to total and maximum number of midges per trap night (Table 2). At the pre-alpine II area, only few individuals were collected at the end of June (n = 50) but the numbers started to rise up around mid-July, with a

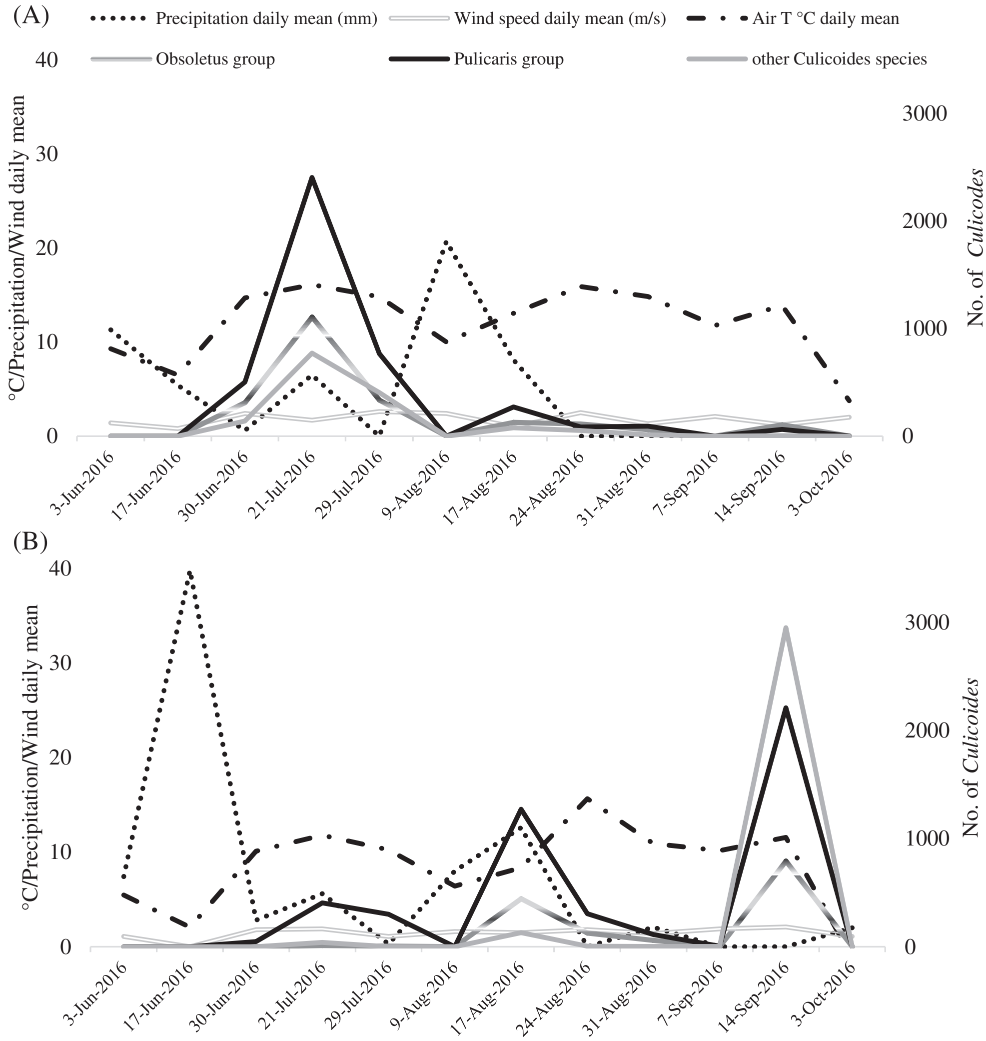

Fig. 2. Abundance of *Culicoides* per night collection during summer 2016 in (A) the pre-alpine I (around 1600 m asl; two sites with one trap each) and (B) pre-alpine II (2030 m asl; one site) areas. Included are all specimens (sorted to species group level, parous *Culicoides* and individuals used for species identification and logistic regression analysis).

first peak mid-August, with dominating Pulicaris group species (n = 1271). Activity was also interrupted at the beginning of August and early September (Fig. 2B). A second and more pronounced peak was observed in mid-September, with the other *Culicoides* species being in a greater number (n = 2952) compared to the Pulicaris group species (n = 2210). In addition, the overall number of *Culicoides* collected at this altitude and the maximum per trap night (nearly 6000 in the night on 14th of September 2016) were higher than at either of the lower altitude farms (Fig. 2B). See Table S2 for the total number of *Culicoides* according to groups collected per night at each farm.

Discussion

There is still a lack of data on seasonal activity of *Culicoides* at higher altitudes in Europe, mainly due to location inaccessibility, livestock movement and funding constraints, and we here present the *Culicoides* prevalence at altitudes between 1500 and 2100 m asl.

The *Culicoides* distribution and lifespan is very much related to temperature and climatic conditions (Carpenter *et al.*, 2009) where dryness and low temperatures could lead to an increase of mortality. The 2016 pre-alpine summer season was quite cool (unexpectedly, snow was recorded in June) comparing to the previous year (Meteoswiss, the Federal Office of Meteorology and Climatology), which most probably determined a lower *Culicoides* abundance in our catches. Such fluctuations, due to temperature variability on species abundance between seasons, are considered normal in the literature (Meiswinkel *et al.*, 2014).

Based on the *Culicoides* species prevalence data presented here, the season in the pre-alpine areas is shorter compared to the one at lower altitudes described by other authors (Ander *et al.*, 2012), although we cannot demonstrate this since our trapping activity started from end of June due to heavy snow and low temperatures.

Indeed, meteorological conditions are known to influence adult midges' activity (Mellor *et al.*, 2000). In a laboratory study done in South Africa, the flight activity of *C. imicola* was reported at temperatures ranging between 16 and 18 °C, whereas in the field was noticed at 19–20 °C (Venter *et al.*, 2019). In another study done in Sweden (Ander *et al.*, 2012) *Culicoides* were collected only when temperatures were above 8 °C.

In our work, the lowest temperatures recorded across all collection nights (see Table S1) when we still catch *Culicoides* were 6.9 °C at the pre-alpine I (14th of September 2016) and 6.6 °C at the pre-alpine II area (17th of August 2016).

Our results acknowledge an adaptation of *Culicoides* to cooler climates as confirmed in the study of Cuéllar *et al.* (2018) where it is suggested that midges' activity started at mean temperatures of just 1 and 3 °C. However, the minimum temperatures recorded in the investigated area during the no collection dates (Table S1) were 0.3 °C at the pre-alpine I area and 3.6 at the pre-alpine II area (9th of August 2016).

Temperatures recorded during the whole *Culicoides*' collection period (June–October 2016) were changing very rapidly from one day to another (Fig. S1) with fluctuations up to 7 °C in mid-July. Unfortunately, there are not any studies describing the effect of temperature on the larval development or life span for *Culicoides* species at high altitudes. How the temperature fluctuations influenced *Culicoides* abundance and distribution are unknown but it had an impact on flight activity and therefore on the numbers collected.

Moreover, lower humidity determined by lack of precipitation did not essentially inhibit flight activity and collection of *Culicoides* at the pre-alpine II area (Fig. 2B), as observed in other studies (Viennet *et al.*, 2012). Most likely the wind speed did not interfere with our collection as the daily mean did not surpass 2.6 m/s in both pre-alpine areas (Fig.2A,B) and it is estimated that more than 3 m/s are required to repress the activity of *Culicoides* (Mellor *et al.*, 2000).

Temperature is also one of the major parameters influencing pathogen transmission by competent vectors. For instance, previous studies (Carpenter *et al.*, 2011) reported that the minimum temperature range required for Orbivirus replication is between 11 and 13 °C.

Since in our study we only recorded 2 days with a daily mean temperature within or above this range, we can assume that the risk of BTV transmission at high altitude is very low despite the presence of *Culicoides* vector species. Indeed, a recent study assessing the susceptibility of pre-alpine *Culicoides* (*C. grisescens* I, *C. grisescens* II, *C. obsoletus*, *C.scoticus*, *C. pulicaris*, and *C. deltus*) to BTV infection failed to confirm virus transmission among the 238 individuals tested (Paslaru *et al.*, 2018). The biting midges exposed to a virus-containing blood meal were incubated at a mean temperature of 19 °C (fluctuating temperature regime 13–25 °C, corresponding to a warm spell at that altitude as given by meteorological stations). Although during the whole 2016 pre-alpine summer season, there were time frames with mean temperatures (Fig. S1, Table S1) above 11 °C up to six or 15 days in a row, this length of time may not be sufficient for a transmissible infection of *Culicoides* vectors (Carpenter *et al.*, 2011). However, nothing is known about the preferred microhabitats and related temperature conditions of *Culicoides* in nature which could significantly affect the likelihood of virus replication and transmission (Verhulst *et al.*, 2020). Recently, it has been shown that mosquitoes indeed have temperature preferences (Verhulst *et al.*, 2020).

From the data presented here, it can be postulated that the vector season (Commission, 2007) starts around the end of June in the pre-alpine I area and middle of August in the pre-alpine II area. However, transhumance is also another important aspect which should be taken into consideration beside the weather conditions. In the investigated area, cattle are moved to pastures at higher altitudes during summer time. For instance, cattle from the Davos Clavadel farm (pre-alpine I, Table 1) were brought to the pre-alpine II farm in late-June. This could explain why the *Culicoides* collection, including parous ones (Figs. 1 and 2), starts later at this higher altitude compared to the pre-alpine I area. Interestingly, although no more cattle were left at the pre-alpine I farm after late-June, we continued to collect (lower amounts of) biting midges. This might be due to the presence of cow manure in the vicinity of the farm, which was not removed during our collection time. Cattle manure has been already described as a potential breeding site for *Culicoides* species from the Obsoletus group (Thompson *et al.*, 2013).

According to the summer prevalence of *Culicoides* groups, species of the Pulicaris group were the most abundant regardless of the area and date of collection followed by Obsoletus (Fig. 2A) except late in the season (14th Sept) with other *Culicoides* species being the most abundant at high altitudes (Fig. 2B). The Pulicaris group includes potential vectors species of BTV and AHSV in Europe (Mellor *et al.*, 1990; Caracappa *et al.*, 2003) such as *C. punctatus* (Caracappa *et al.*, 2003) which can also occur in colder areas (Takken *et al.*, 2008) although it was rarely collected in our study. *Culicoides grisescens* II, which also belongs to this group, was the most abundant species at the pre-alpine II area and it increased among the summer season although it was only collected later (end of July) as previously described for Sweden (Ander *et al.*, 2012). However, in this study it was only referred to *C. grisescens* and not to any of the two cryptic species in particular.

These findings correspond to an earlier study done by our group in different climatic regions of Switzerland (Kaufmann *et al.*, 2012b), where an increased amount of *C. grisescens* II was collected at altitudes of 1560–2130 m asl and identified using mass spectrometry. However, that study did not investigate the whole summer but *Culicoides* were collected only during one single night between August and September, 2010.

Culicoides pallidicornis (belonging to the other *Culicoides* species) which is also known to be the second most abundant one when directly aspirated from sheep and cows (Ayllon *et al.*, 2014), was not plentiful in our catches but still it was the predominant species collected among the other *Culicoides* species, at the pre-alpine I area. This species, which is considered univoltine, being active from mid-May to mid-June (Meiswinkel *et al.*, 2014), was present in our catches at least from late-June to mid-September (Table 3).

The Obsoletus group also includes species that have been proven to be competent vector for BTV (Caracappa *et al.*, 2003; De Liberato *et al.*, 2005) AHSV (Mellor *et al.*, 1990) and SBV (Elbers *et al.*, 2013) viruses in Europe. Among these vectors, two species (*C. obsoletus*, and *C. scoticus*) were constantly collected during the summer season at the pre-alpine I farms, as they are already known as multivoltine species (Meiswinkel *et al.*, 2014), whereas *C. chiopterus* was only collected once and in a very low number (Table 3) although the presence of this species might have been underestimated. Indeed, a study done in the United Kingdom showed that even though *C. chiopterus* was abundant on sheep, it was nearly absent in the light traps situated close by, inferring that light traps catches are not accurately reflecting the proportion of *Culicoides* biting on animals in the field (Carpenter *et al.*, 2008). Viennet *et al.* (2011) obtained similar results in France utilizing drop traps, direct aspiration, sticky cover traps and light traps.

To the best of our knowledge, this is the first study describing the summer species prevalence of *Culicoides* at higher altitudes in Europe, with *C. obsoletus* and *C. grisescens* II being the most abundant ones overall. Although previous works on the occurrence of *Culicoides* species at high altitudes in Switzerland were carried out, species description (by morphology only) was given only for two nights collections in early-June and early-September (Kaufmann *et al.*, 2009) or one collection night by mass spectrometry in August-September (Kaufmann *et al.*, 2012a).

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Daily temperatures (low, high and mean) recorded from May to June 2016 at the pre-alpine I and pre-alpine II areas.

Table S1. Minimum and maximum temperatures registered in the two pre-alpine areas during summer collection in 2016.

Table S2. Total number of *Culicoides* collected per trap/night during summer season 2016 at the three selected farms from the pre-alpine areas, including those used for species identification and parous females.

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The authors declare that they have no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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